Vol. 144, No. 1, 1987 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
April 14, 1987 Pages 422-431

DIVERGENCE OF ANF ANALOGS IN SMOOTH MUSCLE CELL cGMP RESPONSE AND AORTA VASORELAXATION: EVIDENCE FOR RECEPTOR SUBTYPES

Gerald P. Budzik[1], Susan L. Firestone, Eugene N. Bush, Peter J. Connolly[2], Todd W. Rockway, Virender K. Sarin, and William H. Holleman

Division of Cardiovascular Research,
Abbott Laboratories,
Abbott Park, Illinois 60064

Received Feburuary 4, 1987

SUMMARY: ANF analog potencies in stimulating smooth muscle cell cGMP were compared with the ability to relax histamine-constricted rabbit aorta in vitro. ANF[1-28][3], [5-28], [5-27] and Lys-11[5-28] elevated cGMP and were potent vasorelaxants. ANF[7-23] and Lys-11[7-23] were potent cGMP stimulators but 1000-fold weaker relaxants. Tyr-8[5-27] did not stimulate cGMP synthesis or antagonize the response of the other peptides, yet was a potent vasorelaxant. Crosslinking with ^{125}I-ANF identified bands at 150 and 65 KD by SDS-PAGE. ANF[1-28], Lys-11[7-23] and Tyr-8[5-27] blocked crosslinking at low concentration despite disparate activities. These data support the existence of ANF receptor subtypes and suggest that cGMP elevation alone is not sufficient to promote atrial peptide-induced vasorelaxation.

A role for cGMP as a general mediator of vasodilation has been established (2). It has also been suggested that cGMP mediates the effects of atrial natriuretic factor peptides (3). Atrial peptides can raise cGMP in aorta (4,5), smooth muscle cells (6,7), endothelial cells (8-10), kidney (11,12), renal juxtaglomerular (13) and LLC-PK1 (14) cells, adrenal (15,16), and neural cells (17-19).

We evaluated ANF analogs for their ability to elevate cGMP levels from vascular smooth muscle cells and to relax histamine-constricted rabbit aorta.

[1] Author to whom correspondence should be addressed.

[2] Current address: Ortho Pharmaceuticals Corp., Dept. of Medicinal Chemistry, P.O. Box 300, Raritan, NJ 08869.

[3] Atrial natriuretic factor (ANF), [1-28], refers to the sequence of the 28-residue peptide from rat atria (1). Analogs are named relative to ANF[1-28] by noting positions of amino and carboxyl-terminal residues within brackets, []; internal replacements are indicated preceding brackets.

0006-291X/87 $1.50

We have been able to separate the cGMP stimulatory and vasorelaxant activities in unique, synthetic peptides. Our data indicate that receptor-mediated cGMP elevation alone is neither sufficient nor required for ANF-induced relaxation and imply the existence of multiple receptor subtypes.

MATERIALS AND METHODS

Peptides. ANF[1-28], ANF[5-27], Arg-8 vasopressin (AVP), human angiotensin-II (A-II), human adrenocorticotropic hormone (ACTH), and bradykinin were from Peninsula. ^{125}I-ANF (~2000Ci/mmole) was from Amersham. Other ANF analogs were prepared by automated solid-phase synthesis (20), each showing one peak by analytical HPLC. Homogeneity was assessed by amino acid analysis, high field Fourier-transform NMR and FAB mass spectroscopy. Stock peptides were prepared at 0.1-1.0 mM in 0.1 M acetic acid, 0.01% Brij-35 (Technicon) and stored at -20°C.

Rabbit Aorta Vascular Smooth Muscle Cells (VSMC). Cultures were prepared from thoracic aorta explants of 1-1.5 kg female New Zealand white rabbits (21). Cells were propagated in Iscove's Modified Dulbecco Medium (Irvine Scientific) with 10% fetal bovine serum (MA Bioproducts), 25 μg/ml gentamicin at 37°C. VSMC were serially subcultured and used subsequent to passage 3 with no discernable differences observed through passage 16.

Stimulation of cGMP Synthesis. Confluent cells (~57,000 cells/cm^2) in 6-well plates (Costar) were used for time-courses and 48-well plates (Costar) for dose-responses 4-6 days after plating. Growth medium was replaced with 0.5 ml (time-course) or 0.1 ml (dose-response) prewarmed assay medium, RPMI-1640 (GIBCO) with 0.1% RIA-grade bovine serum albumin (BSA, Sigma) and 0.2 mM 1-methyl 3-isobutyl xanthine (MIX, Sigma), and equilibrated 15 minutes at 37°C in moist 5% CO_2 before adding peptide. Incubations of 0-2 hours were terminated by acidification with $HClO_4$ to 1.6% (22). Medium was spun in an Eppendorf microcentrifuge for 5 minutes. The supernatant was neutralized with $KHCO_3$ to 0.27 M, and $KClO_4$ sedimented. cGMP was determined after acetylation by RIA (New England Nuclear).

Crosslinking of ^{125}I-ANF to VSMC. Confluent monolayers in 24-well plates (Costar) were equilibrated at 4°C for 2 hours. Growth medium was replaced with 0.2 ml ice-cold assay medium containing 0.15 nM ^{125}I-ANF (~100,000 cpm/0.2 ml) and unlabelled peptide. After 2 hours at 4°C, medium was removed and the cells washed with 1 ml cold PBS (pH 7.4, Sigma). Disuccinimidyl suberate (DSS, Pierce) was diluted from 50 mM in DMSO to 0.5 mM in cold PBS before adding 0.2 ml to cells. Crosslinking was stopped at 30 minutes with 0.1 M $CH_3CO_2NH_4$. After an additional 5 minutes, the monolayer was washed with 1 ml cold 50 mM CH_3CO_2Na (pH 4.0), 100 mM NaCl followed by 1 ml cold PBS. Cells were dissolved in 0.1 ml SDS gel sample buffer with or without 5% 2-mercaptoethanol and boiled 10 minutes. Samples were run on 9% polyacrylamide gels (23). Dried gels were exposed to Kodak X-Omat AR film with a Dupont Cronex Lightning-Plus Intensifying Screen at -80°C for 4-10 days.

Rabbit Aorta Vasorelaxant Assay. Descending thoracic aortae from 2-3 kg rabbits, anesthetized with Abbott sodium pentobarbital (30 mg/kg), were placed in physiological saline (PSS) containing (mM): NaCl, 119.0; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$, 1.5; $NaHCO_3$, 25.0; KH_2PO_4, 1.2; Na_2EDTA, 0.03; Dextrose, 11.0. Rings (3 mm) were mounted in 2 ml PSS baths at 37°C and attached to Harvard 9529 transducers with 1.6 gm resting tension. The rings were equilibrated 15 minutes, exposed to 10 μM (-)norepinephrine for 2 minutes, and washed with PSS. After a 1 hour PSS re-equilibration with washes every 15 minutes, rings were contracted with 6 μM histamine. Cumulative test peptide additions in 50 mM Na phosphate (pH 7.4), 0.1% BSA were then begun at

half-log concentration increments. Assay standard, ANF[5-28], and vehicle were run for each set of determinations.

RESULTS

The potent vasorelaxant ANF[5-28] induced a rapid cGMP increase in VSMC cultures as shown in Figure 1. Intracellular cGMP was elevated 10-fold within 5 minutes peaking at 170 fm/well and plateauing at 50 fm, 3-fold above basal level, for up to 2 hours. The cells also released cGMP into the medium after a 5 minute lag. Extracellular cGMP increased dramatically to a plateau >500 fm/well after 1 hour. Basal intracellular cGMP in control cultures (-ANF) was unchanged during the entire incubation, and basal extracellular cGMP was undetectable during the same period (Figure 1). Similarly, 1 μM AVP, human A-II, human ACTH or bradykinin had no effect on cGMP. The kinetics of cGMP accumulation were similar to the time course of ANF[5-28] (Figure 1) for all the stimulatory ANF analogs used in this study (data not shown), except that the magnitude of the elevation with each peptide varied as described below.

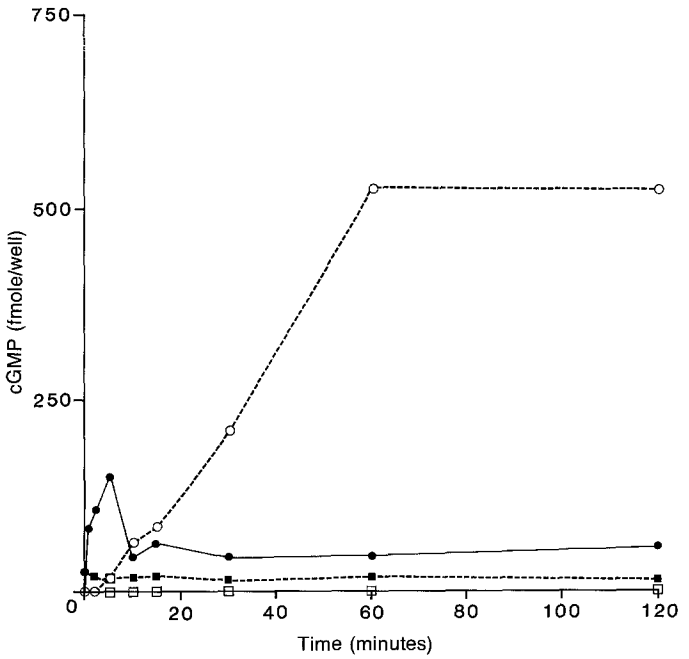

Figure 1. Time-Dependent Stimulation of cGMP with 1 μM ANF[5-28]. VSMC in 6-well plates (~456,000 cells/well) were incubated with or without peptide at 37°C. Incubations were terminated by aspirating the assay medium followed by immediate acidification for extracellular cGMP. Cells were washed rapidly with 1 ml ice-cold PBS and then extracted with 0.5 ml 1.6% $HClO_4$. Each point represents the average of two separate determinations. 1 μM ANF[5-28]: intracellular (●), extracellular (○). Control (-ANF[5-28]): intracellular (■), extracellular (□).

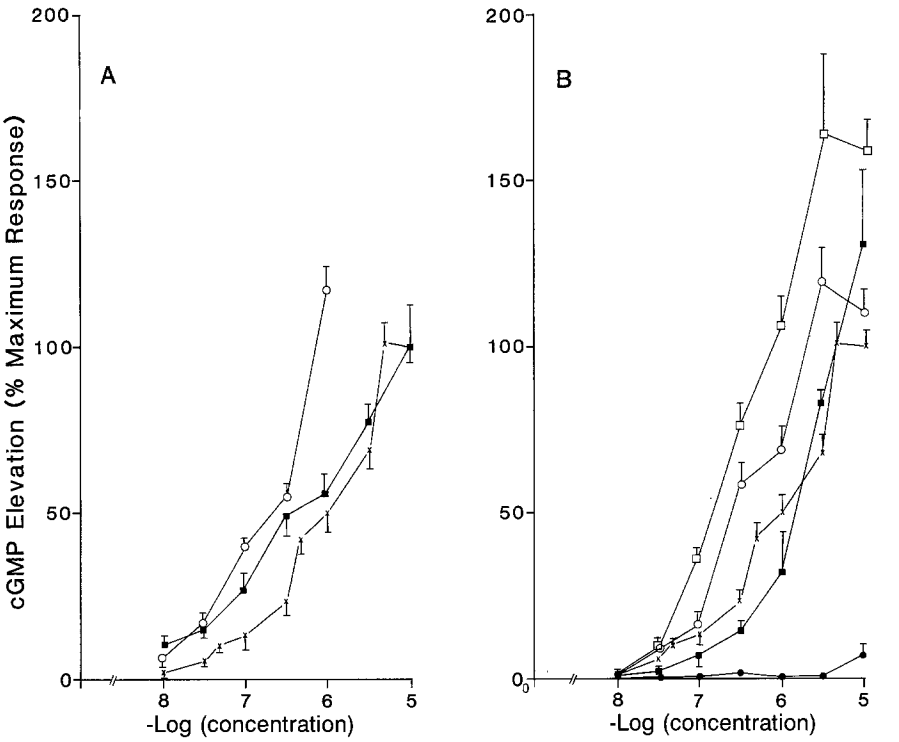

Figure 2. Concentration-Dependent Stimulation of cGMP with ANF Analogs. VSMC in 48-well plates (~57,000 cells/well) were incubated with peptides for 2 hours at 37°C. Incubations were terminated by acidification of complete cultures without separating medium from cells. Data are expressed as % Maximum Response relative to the assay standard, ANF[5-28]. Maximum standard response is set at 100% and all data normalized to it. Each point represents the average of at least four separate experiments. Bars indicate the standard error of the mean. Panel A: ANF[1-28] (○); [5-27] (■); [5-28] standard (X). Panel B: Lys-11[5-28] (□); Lys-11[7-23] (○); [7-23] (■); [5-28] standard (X); Tyr-8[5-27] (●).

As seen in Figure 2A, total cGMP stimulation by ANF[5-28] is concentration-dependent, plateauing at about 20 μM. No reproducible cGMP rise was seen below 10^{-8} M peptide. Concentration-dependent stimulations of cGMP with two other potent agonists are shown in Figure 2A. ANF[1-28], the major plasma form in rats (25), was more potent than [5-28] in the cGMP assay, while ANF[5-27] (26) exhibited a dose-response similar to [5-28].

Figure 2B shows dose-response relationships of four analogs compared to the assay standard. ANF[7-23], a peptide lacking the N- and C-terminal portions of ANF[1-28], produced a cGMP response similar to [5-28]. Replacement of Arg-11 in [7-23] or [5-28] with Lys yielded analogs with greater potency in the cGMP assay than their respective parent peptides. In marked contrast, replacement of Phe-8 in [5-27] with Tyr completely abolished a cGMP response.

Biological potency of each analog was assessed from its ability to relax the histamine-constricted rabbit aorta ring and expressed as pD_2 ±SEM (n). The pD_2 is the negative log of the concentration producing half-maximal relaxation. All analogs tested here produced 100% relaxation at appropriate concentrations. Values of pD_2 for each ANF analog are: [1-28], 9.42±0.10 (4); [5-28], 8.90±0.11 (6); [5-27], 8.81±0.17 (4); [7-23], 6.08±0.07 (6); Lys-11[7-23], 5.95±0.15 (4); Lys-11[5-28], 8.90±0.29 (5); and Tyr-8[5-27], 7.93±0.10 (4). Three peptides [1-28], [5-28] and [5-27], which stimulated cGMP in VSMC (Figure 2A), were potent vasorelaxants. The activities of [7-23] and Lys-11[7-23], however, were reduced 3 orders of magnitude. Nevertheless, these truncated peptides exhibited an equal or greater abililty to stimulate cGMP than [5-28] (Figure 2B). Lys-11[5-28] which had vasorelaxant potency equal to its unmodified parent, and slightly lower than [1-28], was nevertheless as active as the 28-residue peptide in the cGMP assay (compare Figures 2A and B). Replacement of Phe-8 with Tyr in [5-27] slightly reduced vasorelaxation relative to the unmodified peptide but completely abolished cGMP stimulation.

These data suggest the existence of multiple ANF receptor subtypes both coupled and uncoupled to guanylate cyclase. The inability of Tyr-8[5-27] to elevate cGMP could arise if either it bound to receptor but did not activate coupled cyclase or the peptide only had affinity for the uncoupled receptor. These alternatives were addressed by the experiment shown in Figure 3. Concentration-dependent cGMP stimulation by [5-28] and Lys-11[7-23] were assessed in the absence and presence of 3 μM Tyr-8[5-27]. The Tyr-8 analog does not antagonize the dose-response of either potent cGMP-stimulating agonist (Figure 3). Similarly, the Tyr-8 analog had no effect on the concentration-dependent elevation of cGMP by ANF analogs Lys-11[5-28], [5-27] and [7-23] (data not shown). This indicates that Tyr-8[5-27] does not bind to a cyclase-coupled ANF receptor. However, since it is a potent vasorelaxant, it must act through a subtype not mediated by cGMP.

The ANF receptor(s) was identified in VSMC by crosslinking ^{125}I-ANF to cells with DSS and fractionating labelled proteins by SDS-PAGE. Figure 4A shows that proteins at 150 and 65 KD were labelled specifically, and crosslinking was completely blocked above 10^{-9} M ANF[1-28]. Only the 65 KD band remained (Panel A) after reduction with 5% 2-mercaptoethanol prior to SDS-PAGE. Similar results were obtained with Lys-11[7-23] (Panel B) and Tyr-8[5-27] (Panel C). These analogs exhibit high affinity for both molecular weight components, though reduced slightly compared to [1-28]; crosslinking to both bands was blocked above 10^{-8} M. The displacement with each peptide in reduced samples (data not shown) was similar.

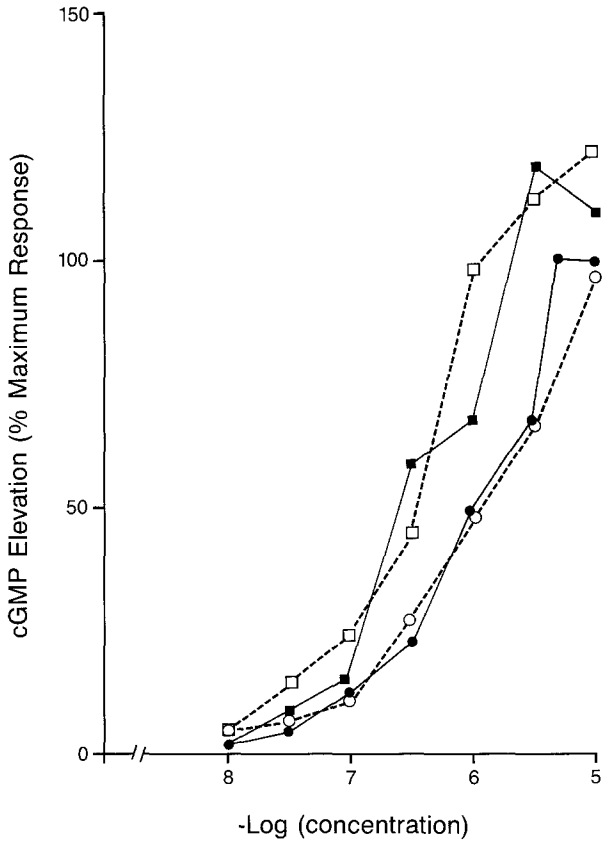

Figure 3. Lack of Inhibition by Tyr-8 ANF[5-27] of Agonist-Dependent cGMP Elevations. VSMC in 48-well plates with increasing concentrations of either ANF[5-28] or Lys-11[7-23] were co-incubated with 3 μM Tyr-8[5-27] for 2 hours at 37°C. Total cGMP was determined as before. Each point represents the average of at least two determinations. ANF[5-28] alone (●), ANF[5-28] + 3 μM Tyr-8[5-27] (○). Lys-11 ANF[7-23] alone (■), Lys-11 ANF[7-23] + 3 μM Tyr-8[5-27] (□).

DISCUSSION

Consistent with observations from many other groups (1-18), we have shown that physiologically potent ANF peptides, [1-28], [5-28] and [5-27], induce dramatic cGMP elevations in VSMC cultures. Others have proposed that the receptor transduction mechanism is triggered when ANF binds to its plasma membrane receptor and activates a functionally-coupled particulate guanylate cyclase (2,3,9-11,15,27-28). A recent report suggests that both activities reside in the same protein (29).

In this study we show that cGMP and vasorelaxant activities can be segregated by different synthetic analogs. ANF[7-23] and Lys-11[7-23] induce cGMP stimulations with equal or greater potency than [5-28]; however, the truncated peptides are 1000-fold weaker vasorelaxants of the rabbit aorta than [5-28].

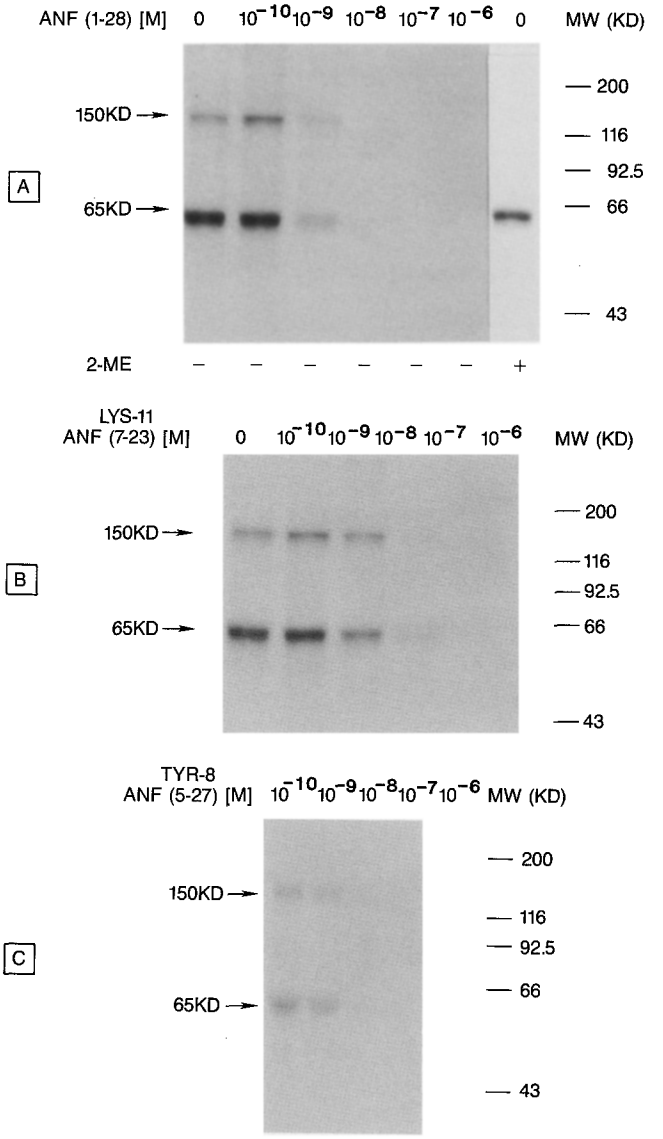

Figure 4. Concentration-Dependent Inhibition of ^{125}I-ANF Crosslinking to VSMC with ANF Peptides. ^{125}I-ANF was bound to VSMC in the presence of increasing concentrations of unlabelled ANF analogs. Cells were washed and exposed to crosslinking reagent as described. Solubilized VSMC were fractionated on 9% polyacrylamide gels, autoradiograms of which are shown. Molecular weights of co-electrophoresed protein standards are indicated. Only the sample shown in Panel A was reduced with 5% mercaptoethanol(2-ME) before electrophoresis; all others were left unreduced. Competing analogs are: panel A, ANF[1-28]; panel B, Lys-11 ANF[7-23]; panel C, Tyr-8 ANF[5-27].

Similarly, Lys-11[5-28] was as potent as [1-28] in the cGMP assay but less active in vasorelaxation. In marked contrast, Tyr-8[5-27] lacks cGMP activity but is almost equally potent to [5-27] in the vasorelaxant assay. These data indicate that an elevation in cGMP level is, by itself, inadequate to mediate

ANF signal transduction. More importantly, Tyr-8[5-27] induces aorta vasorelaxation, yet is incapable of generating a cGMP response in VSMC, indicating the biological event occurs independent of cGMP generation.

The importance of cGMP for ANF signal transduction in other tissues, has also been questioned (30-33). Inhibition of the A-II dependent increase in adrenal aldosterone steroidogenesis by ANF[1-28] is accompanied by cGMP elevation. However, neither 8-bromo cGMP nor dibutyryl-cGMP have any affect on A-II stimulated aldosterone levels (30-32). Furthermore, sodium nitroprusside, a potent cGMP effector, left aldosterone unaffected, and diltiazem inhibited aldosterone production yet did not alter cGMP levels (30). In fact, 8-bromo cGMP is a weak stimulator of aldosterone synthesis (31). In anesthetized dogs, Seymour et al. (33) concluded that elevation of urinary cGMP excretion following direct ANF infusion into the renal artery was not primarily responsible for the renal effects of the peptide. It has also been shown that although ANF induces relaxation of phorbol ester-constricted blood vessels with a concomitant cGMP elevation, relaxation was not stimulated by 0.1 mM 8-bromo cGMP alone (34).

An important factor is frequently overlooked in many studies which attempt to correlate cGMP elevation with the actions of ANF. Half-maximal receptor occupancy and physiological effects are usually observed in the range of 0.1-1 nM ANF (35). However, a threshold ANF level required to elicit even a moderate cGMP response is 5-10 nM (35). This concentration is also at least 10-fold higher than the average circulating ANF level in both rats and humans (36). Based on these discrepancies, it was suggested that cGMP elevation is only a secondary phenomenon following ANF receptor occupancy (35-37) as seen with other hormones (38). Our results in this study support this view.

Crosslinking of ^{125}I-ANF to VSMC specifically labels two components at 150 and 65 KD. The reduction sensitivity of the 150 KD component indicates that it contains at least one 65 KD subunit which seemingly possesses the ^{125}I-ANF binding site. The peptides, [1-28], Lys-11[7-23] and Tyr-8[5-27], block ^{125}I-ANF crosslinking similarly, although each stimulates very different cGMP and vasorelaxant responses. This was unexpected, notably for Tyr-8[5-27] which neither stimulates cGMP nor blocks the response of the other peptides. Whether the two ^{125}I-bands represent different ANF receptor subtypes or simply multimers of the same subtype is still unclear. The crosslinking reagent, DSS, may also be unable to link ^{125}I-ANF covalently to all receptor forms. Variable crosslinking yields have been observed in rabbit aorta membranes with different reagents (39).

Others have suggested that the C-terminal, and to a lesser extent the N-terminal, ANF residues are essential for coupling ANF receptor to guanylate

cyclase in bovine VSMC and endothelial cells (10,40). Our results show this is not the case in all species. We too have found that atriopeptin-I (i.e., ANF[5-25]) is a poorer cGMP stimulator than ANF[5-28] (data not shown) as found in bovine cells (10,40). However, at least in rabbit aorta VSMC, both ANF[7-23] and Lys-11[7-23] are as effective as [5-28] in elevating cGMP, yet lack the complete C- and N-terminal tails of the larger peptide.

Our data strongly suggest that interpretations of a central role for cGMP in ANF signal transduction must be made with caution. Although biologically-potent ANF peptides stimulate cGMP in vivo and in vitro, we have separated the vasorelaxant and cGMP activities in unique, synthetic analogs. This study indicates that cGMP is not the intracellular messenger mediating the vaso-relaxant activity of ANF but is only indirectly related to receptor occupancy and activation.

ACKNOWLEDGEMENTS: The authors thank Ms. Linda D. Artman and Ms. Elizabeth M. Green for performing the rabbit aorta vasorelaxant assays.

REFERENCES

1. Flynn, T.G., deBold, M.L., and deBold, A.J. (1983) Biochem. Biophys. Res. Commun. 117, 859-865.
2. Murad, F. (1986) J. Clin. Invest. 78, 1-5.
3. Hamet, P., Tremblay, J., Pang, S.C., Skuherska, R., Schiffrin, E.L., Garcia, R., Cantin, M., Genest, J., Palmour, R., Ervin, F.R., Martin, S., and Goldwater, R. (1986) J. Hypertens. 4, S49-S56.
4. Winquist, R.J., Faison, E.P., Waldman, S.A., Schwartz, K., Murad, F., and Rapaport, R.M. (1984) Proc. Natl. Acad. Sci. USA 81, 7661-7664.
5. Ohlstein, E.H., and Berkowitz, B.A. (1985) Hypertension 7, 306-310.
6. Harris, D.W., Baker, C.A., Saneii, H.H., and Johnson, G.A. (1985) Life Sci. 37, 591-597.
7. Hirata, Y., Tomita, M., Takada, S., and Yoshima, H. (1985) Biochem. Biophys. Res. Commun. 128, 538-546.
8. Schenk, D.B., Johnson, L.K., Schwartz, K., Sista, H., Scarborough, R.M., and Lewicki, J.A. (1985). Biochem. Biophys. Res. Commun. 127, 433-442.
9. Leitman, D.C., and Murad, F. (1986) Biochim. Biophys. Acta 885, 74-79.
10. Leitman, D.C., Andresen, J.W., Kuno, T., Kamisaki, Y., Chang, J.W., and Murad, F. (1986) J. Biol. Chem. 250, 11650-11655.
11. Ardaillou, N., Nivez, M.P., and Ardaillou, R. (1985) FEBS Lett. 189, 8-12.
12. Tremblay, J., Gerzer, R., Vinay, P., Pang, S.C., Beliveau, R., and Hamet, P. (1985) FEBS Lett. 181, 17-22.
13. Kurtz, A., Bruna, R.D., Pfeilschifter, J., Taugner, R. and Bauer, C. (1986) Proc. Natl. Acad. Sci. USA 83, 4769-4773.
14. Inui, K., Saito, H., Matsukawa, Y., Nakao, K., Morii, N., Imura, H., Shimokura, M., Kiso, Y., and Hori, R. (1985) Biochem. Biophys. Res. Commun. 132, 253-260.
15. Jaiswal, N., Paul, A.K., Jaiswal, R.K. and Sharma, R.K. (1986) FEBS Lett. 199, 121-124.
16. Waldman, S.A., Rapaport, R.M., and Murad, F. (1984) J. Biol. Chem. 259, 14332-14334.
17. Takayanagi, R., Grammer, R.R., and Inagami, T. (1985) Life Sci. 39, 573-580.
18. Friedl, A., Harmening, C., and Hamprecht, B. (1986) J. Neurochem. 46, 1522-1527.

19. Heisler, S., Simard, J., Assayag, E., Mehri, Y., and Labrie, F. (1986) Mol. Cell. Endocrinol. 44, 125-131.
20. Merrifield, R.B. (1964) Biochemistry 3, 1385-1390.
21. Ross, R. (1971) J. Cell Biol. 50, 172-185.
22. Van Haastert, P.J.M., Van Der Meer, R.C., and Konijn, T.M. (1981) J. Bacteriol. 147, 170-175.
23. Laemmli, U.K. (1970) Nature 227, 680-685.
24. Geller, D.M., Currie, M.G., Wakitani, K., Cole, B.R., Adams, S.P., Fok, K.F., Siegel, N.R., Eubanks, S.R., Galluppi, G.R. and Needleman, P. (1984) Biochem. Biophys. Res. Commun. 120, 333-338.
25. Schwartz, D., Geller, D.M., Manning, P.T., Siegel, N.R., Fok, K.F., Smith, C.E., and Needleman, P. (1985) Science 229, 397-400.
26. Currie, M.G., Geller, D.M., Cole, B.R., Siegel, N.R., Fok, K.F., Adams, S.P., Eubanks, S.R., Galluppi, G.R. and Needleman, P. (1984) Science 223, 67-69.
27. Waldman, S.A., Rapoport, R.M., Fiscus, R.R., and Murad, F. (1985) Biochim. Biophys, Acta 845, 298-303.
28. Tremblay, J., Gerzer, R., Pang, S.C., Cantin, M., Genest, J., and Hamet, P. (1985) FEBS Lett. 194, 210-214.
29. Kuno, T., Andresen, J.W., Kamisaki, Y., Waldman, S.A., Chang, L.Y., Saheki, S., Leitman, D.C., Nakane, M., and Murad, F. (1986) J. Biol. Chem. 261, 5817-5823.
30. Matsuoka, H., Atarashi, K., Sugimoto, T., Ishii, M., Sugimoto, R., and Kangawa, K. (1985) Endocrinology (Suppl.) 116, 158.
31. Elliot, M.E. and Goodfriend, T.L. (1986) Fed. Proc. 45, 2376-2381.
32. DeLean, A. (1986) Life Sci. 39, 1109-1116.
33. Seymour, A.A., Blaine, E.H., Mazack, E.K., Smith, S.G., Stabilito, I.I., Haley, A.B., Napier, M.A., Whinnery, M.A., and Nutt, R.F. (1985) Life Sci. 36, 33-44.
34. Lockette, W., and Otsuka, Y. (1986) Hypertension 8, 827.
35. Anderson, J.V., and Bloom, S.R. (1986) J. Endocrinol. 110, 7-17.
36. Ballerman, B.J., and Brenner, B.M. (1985) J. Clin. Invest. 76, 2041-2048.
37. Sagnella, G.A., and MacGregor, G.A. (1986) Trends in Biochem. Sci. 11, 299-302.
38. Goldberg, N.D., and Haddox, M.K. (1977) Ann. Rev. Biochem. 46, 823-896.
39. Vandlen, R.L., Arcuri, K.E., and Napier, M.A. (1985) J. Biol. Chem. 260, 10889-10892.
40. Scarborough, R.M., Schenk, D.B., McEnroe, G.A., Arfsten, A., Kang, L.L., Schwartz, K., and Lewicki, J.A. (1986) J. Biol. Chem. 261, 12960-12964.